Methods: Deqing and Guanyun, two rural Chinese counties in neighboring provinces (zhejiang and Jiangsu), were selected as the field site, where 164 and 187 isolates were collected from the pulmonary TB patients registered in local TB dispensaries during 2004/2005. The susceptibility to 1st line anti-TB drug and Fluoroquinolones was determined by the proportion methods. IS*6110* restricted fragment polymorphism (RFLP) was performed for genotyping and DNA sequencing on hotspot region of *gyrA* gene was performed as well.

Results: Fluoroquinolones resistance was detected in 31 of 351 isolates including 24(6.8%), 11(3.1%) and 10(2.8%) resistant to ciprofloxacin, ofloxacin and levofloxacin respectively. Fluoroquinolones resistance was equally distributed in MDR-TB(15.8% and 11.8%), other combination(8.5% and 9.1%) and 1st line drug susceptible group(6.3% and 7.7%) both in Deqing and Guanyun. And the similar proportion of fluoroquinolones resistance has also been observed in groups of subjects with different social-demographic, clinical and bacteriological features. Mutations in the quinolone resistance determining regions (QRDRs) of *gyrA* were found in 17 of 31 fluoroquinolones resistant isolates in codon 94(45.2%), 90(12.9%) and 74(3.2%). IS*6110*-RFLP identified 2 clusters within fluoroquinolones resistant isolates and 3 clusters composed by both fluoroquinolones resistant isolates and fluoroquinolones susceptible isolates. The geographic distribution of the fluoroquinolones resistant TB patients noted their mainly distributed in central town areas of rural counties both in two counties, with 6(42.9%) from Deqing and 8(47.1%) from Guanyun respectively.

Conclusion: Fluoroquinolones resistance has emerged in *M.TB* circulating in rural China and mainly related to the mutation in QRDR region of *gyrA*. The selective growth of fluoroquinolones resistant strain might be the main cause for the epidemic of fluoroquinolones resistant TB in rural China. And the TB patients from central town areas deserve the special concern for the development of fluoroquinolones resistance.

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59.010

Usefulness of PCR in the differential diagnosis of tuberculosis in paraffin embedded tissues

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Background: Confirmation of differential diagnosis of Mycobacterium tuberculosis (MTB) is important on the finding of granulomatous inflammation that may or may not be associated with infectious agents. Conventional microbiological methods are suboptimal for samples formalin-fixed and paraffin-embedded (FFPE) for histological analysis. The usefulness of PCR for the diagnosis of MTB have been demonstrated with fresh samples, but still requires validation for FFPE. The aim of this study was to compare the diagnosis Neelsen stains.

Methods: We analyzed 110 FFPE (44 lymph nodes, 12 skin, 10 pleura, 10 lung, 5 breast, 4 intestine and 21 other tissues (brain, prostate, uterus, bone marrow, kidney, synovial fluid, larynx, mouth, appendix, among others). FFPE Histologic Groups. According to histologic diagnostic of granulomatous lesion. 1. Granuloma with caseous necrosis ($n = 52$); 2. Granuloma without caseous necrosis ($n = 25$); 3.Granuloma tuberculoid-sarcoidal ($n = 9$); 4. Suppurative granuloma ($n = 7$); 5.Foreign body granuloma/chronic nonspecific inflammation ($n = 17$). FFEP Processing. 2-5 sections of 5 μm of identified lesion were deparaffinized, rehydrated and digested with proteinase K and DNA extracted with a Qiagen-Puregene commercial kit following manufacturer instructions. Quality of DNA was assessed by amplification of a 110 bp beta globin segment. Only FFPE beta globin positive samples were utilized for analysis. Nested IS6110 PCR. Outer and inner primers were utilized to amplyfy a 123 bp segment of IS6110 repetitive insertion of MTB complex genome.

Results: Sensitivities of PCR versus staining for MTB for FFPE were: for granulomas with caseous necrosis, PCR 57.6%, auramine/ZN 19%. For granulomas without caseous necrosis: PCR 24.%, auramine/ZN 12.5%. No positives were found in the other histologic groups for PCR and stains. Clinical validity for PCR was estimated considering as most probable TB granulomas with caseous necrosis and those positive for stains. PCR had: 62% sensitivity, 93% specificity, 88% positive predictive value and 70% negative predictive value.

Conclusion: PCR is a useful complementary technique for diagnosing MTB in FFPE, with sensitivity and specificity considerably greater than auramine/ZN. Nested IS6110 PCR increased the accuracy of histological diagnoses associated with granulomatous tissue response.

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59.011

Geneticdiversity and population structure of *M. tuberculosis strains* circulating in Central Russia

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Background: All of 1200 collected strains with a unique spoligotype (57 of a total 72 spoligotypes) were included into the sample. Among the strains with shared spoligotypes, two to eight epidemiologically unlinked strains were chosen for analysis. The resulting panel of 107 strains should be representative of the diversity of *M. tuberculosis* strains circulating in the Central region of Russian Federation.

Methods: The genetic relatedness of the isolates in the sample was assessed using five genotyping methods: IS*6110*-RFLP, spoligotyping, MIRU-VNTR typing, SNP-based identification of principal genetic groups, PGGs, and SNP clustered groups.

Results: Members of 4 from 6 SCG were identified in the study sample. SCG6/PGG3 strains formed two groups. According to the IS*6110* fingerprint patterns, SCG3/PGG2

was divided on at least 5 groups, including Haarlem strains and all strains with low IS*6110* copy number. MIRU copy number was identified in 12 microsatellite loci. A total of 58 distinct profiles were recognized with 38 unique profiles and 20 clusters comprising from 2 to 23 isolates. Phospholipases C (PLC), have been shown to be involved in *M. tuberculosis* virulence. In this study, we analyzed the frequency of IS*6110* insertions into the phospholipase C operon (*plcABC* genes) among clinical isolates of *M. tuberculosis* from Central Russia. Of the 107 isolates analyzed, 47 contained an IS*6110* insertion. In 45 of the 47 isolates, the IS*6110* element was inserted at the same position and in the same orientation within the *plcA* gene as determined by sequencing. The stable association of the Ins2 IS*6110* insertion in the *plcA* gene with the group supports the hypothesis of their clonal expansion from the common progenitor. The MDR TB level in previously treated patients in prison hospital approaches to 100% and exceeds 50% among civilians. Rate of M tuberculosis strains resistant to RIF INH and KAN was extremely high.

Conclusion: These data support the assumption that a parental strain had been circulating in the environment for a prolonged period of time and had been split into a number of variant strains after acquisition of resistance to many antituberculosis drugs.

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59.012

Evaluation of IS6110 PCR, BACTEC and conventional methods in rapid diagnosis of extra pulmonary tuberculosis cases attending two tertiary care hospitals in North India

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Background: In developing countries the diagnosis of extra pulmonary tuberculosis (EPTB) with conventional diagnostic tools is a major challenge. EPTB encounters many problems like pauci-bacillary nature, inadequate sample volume. All the limitations reflect in the poor contribution of conventional bacteriological technique in the establishment of diagnosis of EPTB. The objective was to evaluate IS6110 PCR for detection of *Mycobacterium tuberculosis* complex for diagnosis of Extra Pulmonary Tuberculosis cases attending tertiary care hospitals.

Setting: Department of Pulmonary Medicine, C.S.M Medical University, Lucknow (Erstwhile King George Medical University) and Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India.

Methods: Specimens from sixty clinically suspected cases of EPTB were processed for Mycobacteria by conventional and BACTEC culture for *M. tuberculosis complex*. All the samples were also processed for PCR amplification with primers targeting 123 bp fragment of insertion element IS6110 of *M. tuberculosis* complex.

Results: Out of 60 cases, 47 (79%) were newly diagnosed; 13 (21%) cases were previous treated cases. Forty five (75%) were male and fifteen were (25%) female. 11/60 (18.3%) were positive for AFB by ZN staining. BACTEC culture was positive in 23/60 (38.8%) and IS6110 PCR was positive in 37/60 (61.6%) cases.

Conclusion: IS6110 PCR can be highly useful in establishment of diagnosis of EPTB.

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59.013

Diagnosis of latent tuberculosis infection by using the QuantiFERON-TB Gold in-tube test in children whose household contact has contagious pulmonary tuberculosis disease

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Background: The QuantiFERON-TB Gold In-Tube test (Cellestis, Carnegie Victoria, Australia) was approved for the diagnosis of latent tuberculosis infection. Although it has been shown to be sensitive and specific in adults, limited data are available on its performance in children.

Methods: This was a case-control study of BCG-vaccinated children younger than 15 years of age in Seongnam, South Korea. We compared tuberculin skin test and QuantiFERON-TB In-Tube test results in children who had exposed to household with contagious pulmonary tuberculosis disease within the past 3 months. Controls with no known exposure history for *Mycobacterium tuberculosis* were matched in a 1:1 ratio to cases according to age and gender.

Results: Among the 27 children with a history of exposure to a household with contagious pulmonary tuberculosis disease, 21 (78%) had positive results for the tuberculin skin test, whereas 8 (30%) had positive results for the QuantiFERON-TB In-Tube test. Among the 27 children with no known exposure history for *M tuberculosis*, none had positive results for both tuberculin skin test and QuantiFERON-TB In-Tube test. There was moderate concordance between tuberculin skin test and QuantiFERON-TB In-Tube test results ($\kappa = 0.43$).

Conclusion: The QuantiFERON-TB In-Tube test is a specific test for diagnosis of latent tuberculosis infection in children whose household contact has contagious pulmonary tuberculosis disease. Additional studies are needed to further assess the utility of the QuantiFERON-TB In-Tube test in the children with high risks of exposure to *M tuberculosis*.

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